ANESTHESIOLOGY

Nitric Oxide Story

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Inhaled Nitric Oxide in Persistent Pulmonary Hypertension of the Newborn. By Roberts JD, Polaner DM, Lang P, and Zapol WM. Lancet 1992; 130:435–40. Reprinted with permission.

Abstract

NO has vasodilatory effects on the pulmonary vasculature in adults and animals. We examined the effects on systemic oxygenation and blood pressure of inhaling up to 80 parts per million by volume of NO at fraction of inspired oxygen 0.9 for up to 30 min by six infants with persistent pulmonary hypertension of the newborn. In all infants, this treatment rapidly and significantly increased preductal oxygen saturation; in five infants, postductal oxygen saturation and oxygen tensions also increased. Inhalation of NO did not cause systemic hypotension or raise methemoglobin. These data suggest that low levels of inhaled NO have an important role in the reversal of hypoxemia due to persistent pulmonary hypertension of the newborn.

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Inhalation of NO is now recognized as a successful and innovative therapy that has been used to treat an estimated 500,000 Americans. When first proposed in 1990, NO was considered to be radical and dangerous. How—over the course of more than 25 yr—did inhaling low doses of what was once considered a poisonous gaseous pollutant become a widespread and life-saving therapy that is Food and Drug Administration–approved to treat term hypoxic "blue babies"?

Warren M. Zapol, M.D.

After my surgical internship, I spent 3 yr working at the National Institutes of Health in the National Heart Institute (Bethesda, Maryland) for an inventive genius: Theodor Kolobow, M.D. As a medical student at Case Western Reserve University, Cleveland, Ohio, Dr. Kolobow invented the first efficient and disposable membrane artificial lung; I received a U.S. Public Health Service commissioned officer post to apprentice as his research fellow from 1967 to 1970. Together we studied and pioneered the field of extracorporeal membrane oxygenation, using awake newborn lambs as our subjects and developing novel cannulae and roller pumps. In 1970 we studied extracorporeal membrane oxygenation therapy of respiratory failure in premature infants in Washington, DC.

I became an anesthesia resident at Massachusetts General Hospital, Boston, Massachusetts, in 1970 and have remained on staff at Massachusetts General Hospital for all the subsequent years. My research during the first 18 yr at Massachusetts General Hospital, from 1970 to 1988, was devoted to extracorporeal membrane oxygenation studies and examining the marked pulmonary vascular changes of the lethal syndrome of acute respiratory distress syndrome in both adults and children. Thanks to the wise guidance of Claude Lenfant, M.D., then the director of the National Institutes of Health, Heart, Lung and Blood Institute (Bethesda, Maryland), and Susanne Hurd, Ph.D., the director of the Lung Division, my colleagues at Harvard University, Boston, Massachusetts, and I were supported for more than a decade by a Specialized Center of Research (Massachusetts General Hospital) in acute respiratory distress syndrome. None of the subsequent inventions would have occurred

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without this stable, long-term support. Over those years I studied both the human and lamb pulmonary circulation in health and disease. We began with hemodynamic investigations (with Swan-Ganz pulmonary artery catheters [Edwards Life Science, USA] and flowmeters), then histological studies, and progressed to studies of two newly discovered important mediator systems that control constriction and dilation of the lung's vessels—prostaglandins and later NO. We reported that pulmonary vasoconstriction was a major and diffuse process occurring in acute respiratory failure, causing acute pulmonary hypertension, and accentuating the formation of pulmonary edema.[1] We did not know how to cure this high blood pressure in the normally low-pressure pulmonary circulation. We studied intravenous delivery of vasodilator drugs including prostaglandins, prostacyclin, and nitroprusside (later identified to be an NO donor compound). Given intravenously by infusion, these drugs dilated the lung's blood vessels and lowered the pulmonary vascular resistance and pressure, but unfortunately these drugs also dilated the body's blood vessels, lowering the systemic arterial blood pressure and causing shock and impairing contraction of the heart. In addition, intravenous vasodilating agents reduced the ability of the lung's vessels to divert blood flow away from injured lung areas; vessels in collapsed and fluid-filled regions normally constrict in an attempt to force blood flow toward better ventilated regions. Unfortunately, intravenous vasodilators impaired this important property of lung vessels, making patients with injured lungs, given these nonselective vasodilators, more systemically hypoxic.

By the middle 1980s, critically important discoveries that led to the Nobel Prize in Physiology or Medicine in 1998 were tied together in a new understanding of vascular biology. Ferid Murad, M.D., Ph.D., a professor at the University of Virginia, Charlottesville, Virginia (and once a Massachusetts General Hospital Medicine house officer), identified cyclic guanosine monophosphate as an intracellular second messenger in various cells and tissues of the body. Much was known about cyclic adenosine monophosphate at this time, but a precise biologic role for cyclic guanosine monophosphate remained unclear. In the 1980s, Robert Furchgott, Ph.D., a pharmacologist at the State University of New York Downstate, Brooklyn, New York, reported that when vascular endothelial cells were stimulated by acetylcholine, they produced a substance that he called "endothelium-derived relaxing factor." The final piece of the puzzle was placed by Louis Ignarro, Ph.D., a pharmacologist at the University of California, Los Angeles, Los Angeles, California. Dr. Ignarro demonstrated that endothelium-derived relaxing factor was NO, a gas produced from arginine by endothelial (and other) cells, and that NO acted as a mediator to promote vasodilation. As part of his convincing proof of the identity of endothelium-derived relaxing factor, Dr. Ignarro demonstrated that adding hemoglobin could block the vasodilatory reaction of blood vessels when exposed to either endogenous or exogenously added NO. Because hemoglobin binds to NO with high avidity, hemoglobin acts as an NO sink, and blocks the transmission of NO (the vasodilatory signal) from the endothelial cell to the smooth muscle cell.

The identification of endothelium-derived relaxing factor as NO was an earth-shaking leap forward and spawned an enormous number of advances in immunology, neurobiology, and vascular biology. NO was rapidly proven to be a ubiquitous and important mediator in the nervous, immune, and vascular systems of the human body. In the 1980s, NO was classified as a highly toxic gas, which one would hardly leap to use in physiologic studies. Indeed, it was understood that NO rapidly oxidized in air to nitrogen dioxide, a far more toxic gas, and that when nitrogen dioxide was inhaled it hydrated to nitric acid, a key component of acid rain that was known to injure the lung. It is no surprise that each cylinder of NO that I bought in 1988 was stamped with a skull and crossbones, together with a label detailing the toxic properties of inhaling NO, as well as its ability to oxidize and injure the lung, skin, and eyes. Special stainless-steel pressure regulators were required for NO usage, as ordinary metals would oxidize when exposed to this noxious gas.

About this time, I was being recruited to the University of California, Los Angeles, to become Professor and Chairman of the Anesthesiology Department, and during my visits to the University of California, Los Angeles, I met Dr. Louis Ignarro. Although I ultimately remained at Massachusetts General Hospital, Lou's warm, exuberant personality and obvious insight and brilliance in NO research directed my focus to NO. During one of my visits to Los Angeles, I noticed that the *Los Angeles Times* weather forecast included statistics on the airborne levels of carbon monoxide, nitrogen dioxide, sulfur dioxide, and particulates, informing its readers of the levels of air pollution in the Los Angeles valley, which was plagued by emissions from the large numbers of cars, buses, and combustion engines. I reasoned that ambient nitrogen dioxide, the product of atmospheric oxidation of NO, must have been produced by internal combustion in cars and buses. Therefore, people driving cars and trucks had to be breathing NO in cities, in tunnels, and perhaps even greater levels at rush hour.

At this point I was ready to perform an animal experiment, and I was lucky to work with Claes Frostell, M.D., Ph.D., an intelligent, talented Swedish research fellow–anesthesiologist from the Karolinska Institute, Stockholm, Sweden. Our dream hypothesis was that after NO diffused through the lung, it would dilate the pulmonary vessels in ventilated regions, and then would be annihilated by its rapid reaction with circulating hemoglobin, and therefore not cause systemic vasodilation. The resulting nitrosyl-hemoglobin would rapidly decay to nitrite and nitrate. Our fears were twofold: that we would rapidly convert the circulating hemoglobin to methemoglobin, killing the animal because methemoglobin cannot carry oxygen; and that we would destroy the lung by inhaling oxidant gases (NO and nitrogen dioxide). We knew that the system we developed

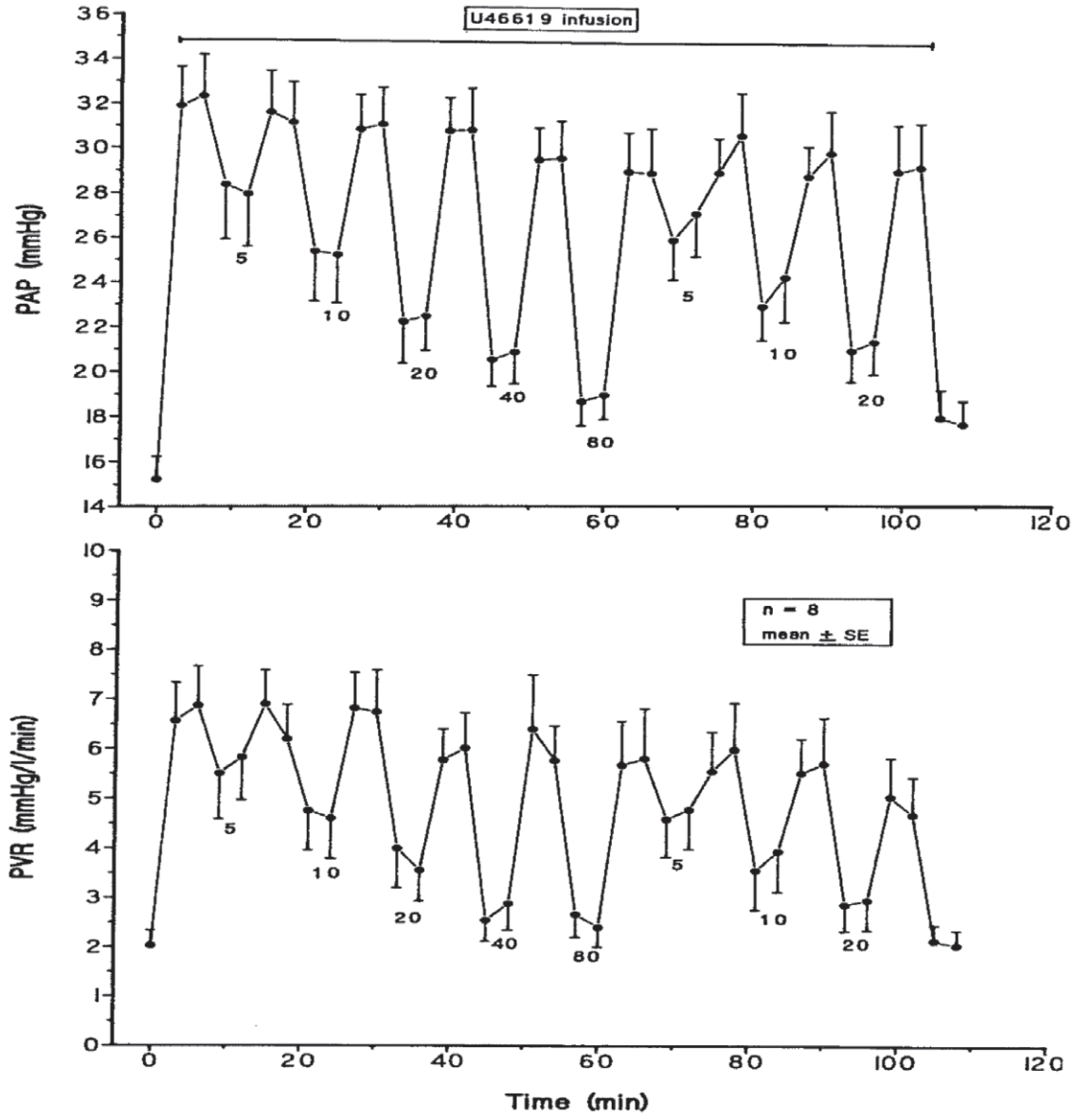

Fig. 1. Plots of mean pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR) during a continuous infusion of U46619. Lambs breathed various levels of NO (5 to 80 ppm) at fraction of inspired oxygen 0.6 for 6 min, then breathed a gas mixture at fraction of inspired oxygen 0.6 for 6 min without NO (n = 8, mean±SEM). Reprinted with permission from Frostell C, Fratacci MD, Wain JC, Jones R, Zapol WM: Inhaled nitric oxide: A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. Circulation 1991; 83:2038–47. Copyright © 1991 Wolters Kluwer Health.

for continuously breathing NO—mixing NO in nitrogen with a continuous stream of oxygen-containing gas—had to avoid excessive nitrogen dioxide production. Before the first lamb experiment, we opened the lab windows, and for added protection, we each wore a gas mask to avoid inhaling the gas mixture we gave the lamb. We induced pulmonary hypertension in an awake, monitored lamb by infusing the thromboxane analog U46619 and tested the ability of low levels of gaseous NO to lower the pulmonary artery pressure.[2] Within a few minutes we knew it would be a "knock your socks off" first experiment. After induction of pulmonary hypertension with U46619, we had the awake lamb breathe 80 ppm NO in 60% oxygen *via* a tracheostomy; within 30 s we witnessed selective pulmonary vasodilation. The pulmonary artery resistance and pressure fell and, most important, the systemic arterial pressure was unchanged (fig. 1). After turning off the gas (while continuing the U46619 infusion) we found that the

pulmonary artery pressure and vascular resistance increased to the pretreatment levels (fig. 1). We measured the blood methemoglobin levels: after 10 min breathing 80 ppm NO, they were under 5%, a very tolerable level. Inhaled NO had produced selective pulmonary vasodilation without reducing the systemic vascular resistance or blood pressure; we had not produced significant methemoglobinemia, and eventually we would show that several hours of breathing 80 ppm NO did not produce lung injury in our lambs.

Before this new therapy could see prime time, it would need to be tested in humans. I recruited a young anesthesiologist and neonatologist, Jesse D. Roberts Jr., M.D., who had just finished the Massachusetts General Hospital anesthesiology chief residency with distinction. Jay had studied surfactant therapy in babies, and was fascinated by our early sheep studies. Together Jay and I wrote the human studies protocols for treating babies with primary pulmonary hypertension of the newborn and children with congenital heart disease. Our proposals were approved by the Massachusetts General Hospital Subcommittee on Human Studies, and so we began the first clinical trials of inhaled NO in pediatric patients.

One of the first babies we tried to "salvage" with inhaled NO was the most successful and unforgettable. This "blue baby" had primary pulmonary hypertension of the newborn and a low arterial oxygen pressure (PaO_2 was 40 mmHg on 100% oxygen with positive pressure ventilation). As we set up our apparatus, the surgeons were preparing and draping the baby for extracorporeal membrane oxygenation cannulation of major vessels. We carefully added stepwise increments of NO to the infant's inhaled gas. The response was stunningly quick. The pulse oximeter showed that the blood oxygen saturation rose from about 50% to 90% within 2 min. The blood oxygen levels kept rising as we gave the baby increasing NO doses to 80 ppm.[3] By this time, we had a pink baby! With a falling partial pressure of carbon dioxide in arterial blood, less mechanical ventilation of the lung was needed to achieve gas transfer for the baby. The most remarkable effect was that the lung's dilation appeared to be selective, so that the baby's blood pressure did not fall while breathing NO (fig. 2).

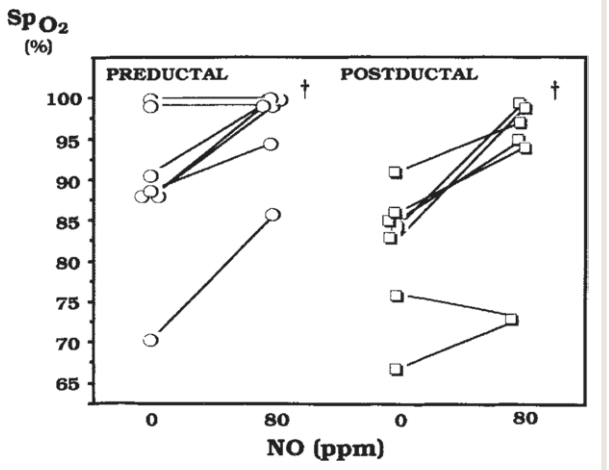

Fig. 2. Change of preductal (○) and postductal (□) oxygen saturation (Spo_2) in infants with primary pulmonary hypertension of the newborn before and during inhalation of 80 ppm NO at fraction of inspired oxygen 0.9. Reprinted with permission from Roberts JD, Polaner DM, Lang P, Zapol WM: Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. Lancet 1992; 340:818–9. Copyright © 1992 Elsevier.

After this triumph, more successful baby trials followed: "blue" babies with primary pulmonary hypertension of the newborn were transferred from nurseries around the Boston area to our Neonatal Intensive Care Unit for NO therapy. In parallel studies, Jay Roberts and Peter Lang, M.D., a pediatric cardiologist then at the Massachusetts General Hospital, began studies of inhaled NO in children with congenital heart disease and pulmonary hypertension. The absence of side effects and the convincing, apparently life-saving, results made the Massachusetts General Hospital Human Studies Subcommittee more comfortable with NO inhalation therapy, and they permitted us to administer NO for longer periods. It was hard to argue with a therapy that appeared to save lives with little added risk.

After our pioneering studies of turning blue babies to pink babies were confirmed and published in 1992 by Steven H. Abman, M.D., and John P. Kinsella, M.D., at the University of Colorado in Denver, Colorado, pediatricians clamored for NO to treat hypoxic newborns. There were more than 100 compassionate use protocols approved in dozens of newborn intensive care units around the United States and abroad. The National Institutes of Health initially sponsored two large trials of inhaled NO in hypoxic newborns. Over the years of study, it became harder to enroll babies; the pediatric community wanted to use NO. In December of 1999, after reaching a positive outcome in the last large randomized trial of inhaled NO in 248 near-term infants with primary pulmonary hypertension of the newborn, the Food and Drug Administration approved inhaled NO as a drug.

In recent years, the use of inhaled NO has become routine in near-term babies with persistent pulmonary hypertension; indeed, we believe that approximately 150,000 babies have inhaled NO in the United States. In addition, about 350,000 adults and children have inhaled NO for off-label uses, including the treatment of pre- and postoperative pulmonary hypertension associated with congenital and adult heart disease, acute respiratory distress syndrome in children, and pulmonary hypertension in adults undergoing cardiopulmonary bypass. Further, we know of significant areas remaining for basic laboratory investigation of NO, including methods to load the body's stores with NO to allow the transfusion of artificial red cell substitutes, which are vasoconstrictors.

Generating NO by Pulsed Electrical Discharge from Air

NO inhalation therapy requires NO/nitrogen gas cylinders and a cylinder distribution network, a complex delivery device to regulate NO, nitrogen dioxide, and oxygen concentrations, and trained respiratory therapists. For many hospitals, inhaled NO is one of the most expensive drugs (at $14,000 per infant) used in neonatal medicine. Because of the complexities and expense of delivering NO, this treatment is not available in many parts of the world and is not practical for outpatient use. Several approaches have been used to produce NO for biomedical purposes, including chemical methods and electrical systems. However, these reactions produce large amounts of toxic byproducts, such as nitrogen dioxide and ozone, and therefore require complex purification systems. In the 1980s, the National Aeronautics and Space Administration (Houston, Texas) flew a plane through the path of a lightning bolt, and measured considerable NO and nitrogen dioxide in the bolt's path. This observation gave me the idea to make NO with an electric spark. Our research group at Massachusetts General Hospital designed, developed, and tested a lightweight, portable, and economic NO generator system that uses pulsed electrical discharges to produce NO. Our NO generator produces NO for inhalation in a therapeutic range (5 to 80 ppm) at gas flow rates of 0.5 to 5 l/min. Iridium electrodes were found to be superior to stainless steel, nickel, carbon, and tungsten electrodes in that they produced the least amount of nitrogen dioxide during NO production. The small amounts of potentially toxic gases that were produced in the electrically generated plasma were removed by a small (12-g) inline calcium hydroxide scavenger. In lambs with acute pulmonary arterial hypertension, breathing electrically generated NO reduced pulmonary arterial pressure as effectively as NO diluted from a conventional cylinder.[4] To save energy, reduce the consumption of the scavenger, and preserve the electrodes, we improved the NO generator to sense and be triggered by inspiratory flow and produce NO only during inspiration. The newly developed NO generator can be installed in series with the ventilator or can be used to inject NO into the airway *via* a transtracheal catheter.

Prolonged use of the NO generator resulted in erosion of the surface of the electrodes, potentially introducing contaminating metal particles into the gas stream.[5] We used quadrupole mass spectroscopy to show that a single high-efficiency particulate air filter was sufficient to remove all metal particles from the effluent gas. Mice in a chamber breathed electrically generated NO, 50 ppm in air for 28 days, without developing pulmonary inflammation or structural changes; no trace metals were detected in the lungs of these mice.[5]

Berra *et al.*[6] tested the electric NO generator on six healthy volunteers and six patients with chronic pulmonary hypertension. Each subject breathed 25 ppm of electrically generated NO for 10 min, and no adverse effects were detected. In patients with chronic pulmonary hypertension, the acute pulmonary hemodynamic effects of electrically generated NO were similar to those measured using NO obtained from commercially available cylinders.[6]

To develop an even lighter and smaller NO generator—for example, to be used in newborns—we designed a miniaturized version of the prototypic NO generator, designated the "mini-NO generator."[7]

In summary, electric plasma NO generation produces therapeutic levels of NO from air, with scavenging and filtration systems that effectively eliminate toxic gas and metallic impurities from the effluent gas. The device provides safe, efficient, and economic NO generation, which will expand the applications of NO therapy to hospitalized and ambulatory pediatric and adult patients around the world.

I have no doubt that there will be more conditions for which inhaled NO will prove to be a valuable, perhaps a life-saving, therapy. Patients will benefit from these new discoveries, just like babies with primary pulmonary hypertension of the newborn benefited from the first clinical breakthrough with inhaled NO.

Competing Interests

Dr. Zapol is an inventor on patents filed by Massachusetts General Hospital, Boston, Massachusetts, on the electric generation of NO. He is on the scientific advisory board of Third Pole Inc., Arlington, Massachusetts, which has licensed patents on NO generators from Massachusetts General Hospital. He does not hold equity in the company.

Correspondence

Address correspondence to Dr. Zapol: Anesthesia Center for Critical Care Research, Massachusetts General Hospital, 55 Fruit Street, Boston, Massachusetts 02114. wzapol@mgh.harvard.edu. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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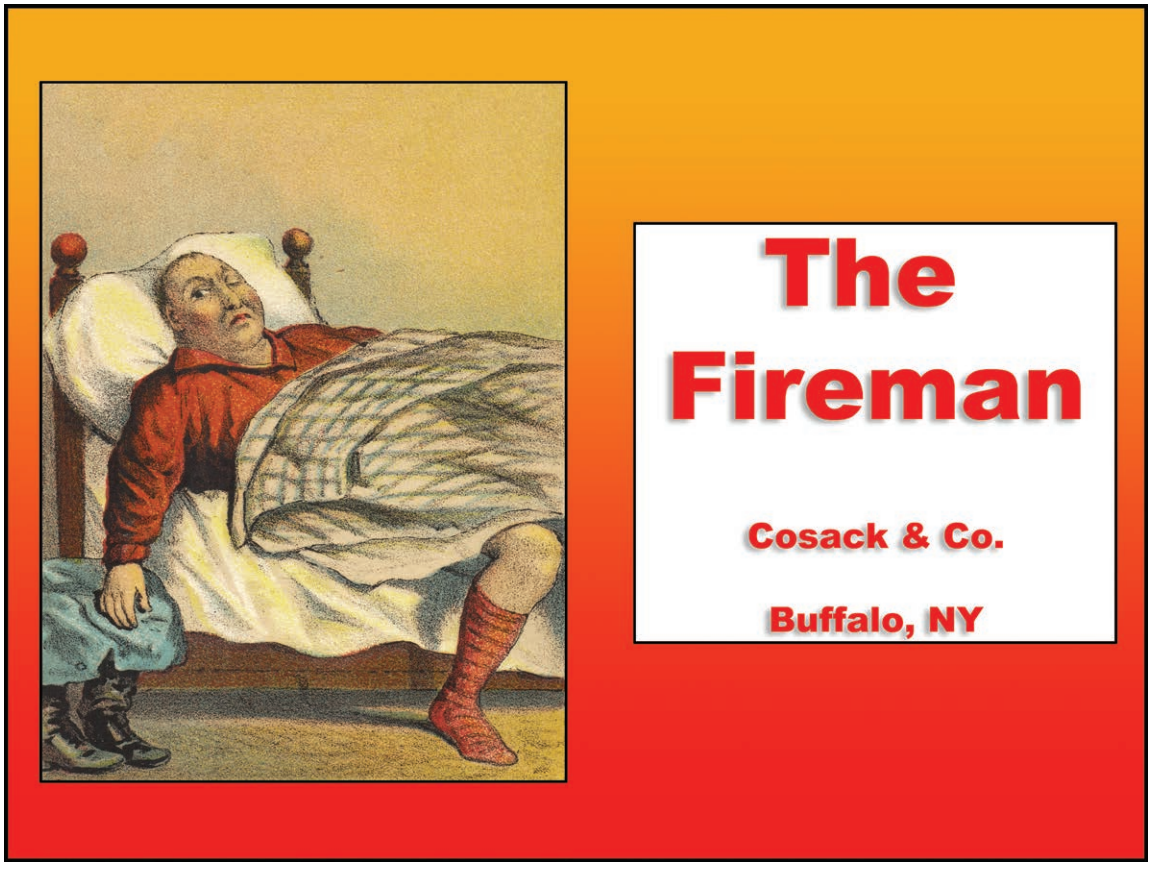
ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

The On-call Anesthesiologist: A Physiological Fireman?



Cosack & Company's 1881 image of *The Fireman* depicts a partially dressed rescuer prepared to leap out of bed—with his right foot planted on the floor and his right hand resting on his trouser-covered fire boots. Ready to launch from sleep at a moment's notice, the fireman reminds me of the on-call anesthesiologist. Rather than extinguishing flames, the physician-anesthetist is prepared to battle the heat of malignant hyperthermia. Whether racing off to command an airway or to administer unscheduled anesthetics for ambulanced traumas, "takeback" open-heart cases, or emergency C-sections, the on-call anesthesiologist, like Cosack's fireman, often sleeps less than restfully…. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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